

ATTEMPTS TO SIMULATE THE ASSOCIATION BETWEEN GEOMAGNETIC ACTIVITY AND SPONTANEOUS SEIZURES IN RATS USING EXPERIMENTALLY GENERATED MAGNETIC FIELDS¹

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Summary.—In three separate experiments, chronic epileptic male rats ($n_s=24$, 24, and 4) were exposed to experimental magnetic fields whose intensities were either constant or varied. The ripple frequency was always 7 Hz. Only exposure to a field with a constant intensity (700 nT) appeared to inhibit occurrence of the seizures. Although exposure to a field configuration whose strength increased and decreased by fixed increments during the hour before feeding did not affect the incidence of seizures, exposure to these fields for 5 min. only once per hour (increments of 15 to 20 nT for 30 sec. to a maximum of 70 nT) during the previous night between midnight and 0800 hr. increased the proportion of seizures following the presentation of food. An explanation is given for the persistent antithetical effects of experimental magnetic fields and geomagnetic activity upon seizure phenomena.

Rats in which seizures have been induced by a single subcutaneous injection of lithium chloride (3 mEq/kg) and pilocarpine (30 mg/kg) become chronically epileptic. The occurrence of overt seizures and the occasional sudden cardiac death (Bureau & Persinger, 1992; Persinger, 1995b) have been associated with sudden increases in global geomagnetic activity, especially when the average daily antipodal index (aa) exceeded 20 to 25 nano-Tesla (20 to 25 gamma). Enhanced concordance between the increased proportion of limbic seizures within this population of epileptic rats and geomagnetic activity occurs when additional food is delivered once per day just before onset of the night cycle (Persinger, 1995a).

The proposed neuromechanism is that the central nucleus of the amygdala is disinhibited from normal intra-amygdaloid and insular control due to the seizure-induced brain injury (Bureau & Persinger, 1992). Because predictable, periodic presentation of food can be a zeitgeber for the release of both adrenocorticotrophic hormone and corticosterone (in rats), we presumed that the major controlling neuropeptide for this sequence, corticotrophin-releasing factor, might also be secreted. This neuropeptide has epileptogenic properties and may be inhibited by normal levels of the intrinsic antiepileptic compound melatonin.

Toroidal experimental magnetic fields whose intensity and direction are

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generated by transients during successive 1-min. periods of activation and deactivation suppress the concentration of melatonin within the pineal organ as well as the activity of the synthesizing enzyme of this serotonin derivative (Lerchl, Honaka, & Reiter, 1991). If enhanced geomagnetic activity (particularly during the previous night) suppresses synthesis of melatonin in a comparable manner, then the observed increase in the proportion of seizures within epileptic rats may be significantly affected by the "conditioned elevation" in the epileptogenic corticotrophin-releasing hormone because of the transiently reduced capacity (or effectiveness) of the endogenous anticonvulsant melatonin.

Most attempts to simulate the association between enhanced geomagnetic activity and increased proportions of either limbic seizures or their correlates have produced the opposite effects, i.e., dampened seizure activity (Bureau & Persinger, 1995). These experiments involved brief exposures to magnetic fields whose strengths ranged between 10 microTesla and 1 milli-Tesla. Geomagnetic activity, which is crudely measured by indices of amplitude whose average values rarely exceed 100 nanoT (and do not reveal the information structure of the variations, i.e., complex patterns of frequencies), can persist for hours.

This study consisted of three separate experiments that attempted to simulate the association between the enhanced incidence of spontaneous seizures in chronic epileptic rats in response to the synchronized, daily presentation of food in free-fed (*ad libitum*) settings. We employed either constant or shifting amplitudes of the experimental field whose ripple frequency was always 7 Hz. This frequency was selected because of its prominent occurrence in nature as the (Schumann) fundamental resonance (Matsushita & Campbell, 1967) of the earth-ionosphere wave guide and its spectral dominance within the continuum of limbic (hippocampal) neuroelectrical activity.

The experiments were designed to obtain data that might differentially support one of two models. The first is that magnetic fields applied just before the conditioned feeding sequences should suppress the residual daytime melatonin (or its correlates) and increase the proportion of seizures following the presentation of the food stimulus. The second model is very similar to that proposed by Schiff, Jerger, Duong, Chang, Spano, and Ditto (1994), who found that the paroxysmal or periodic characteristics of a spontaneously bursting neuronal network such as the CA3 to CA1 input within the hippocampus can be influenced by the pulse-pattern of the electrical stimulation to hippocampal slices. Our working hypothesis has been that disruption of the *process* of gradually exacerbating recruitment of neurons (during the several tens of minutes preceding an overt seizure) would require little energy during these initial stages but would decrease the probability that the discharges would generalize to overt motor displays.

METHOD

All of the rats were male, 80- to 90-day-old Wistar albinos (obtained from Charles River) and had exhibited rearing, rapid forepaw clonus, and falling in response to a single subcutaneous injection of lithium chloride (3 mEq/kg) followed 4 hr. later by 30 mg/kg of pilocarpine. To facilitate survival, 25 mg/kg of acepromazine (Atravet) had been injected 30 min. after the onset of the forepaw clonus (Persinger, Bureau, Kostakos, Peredery, & Falter, 1993). Before assignment to a particular experiment, none of the rats had been exposed to any experimental treatment involving magnetic fields. All field strengths were measured with a Metex M-3800 digital multimeter and sensor [Electric Field Measurement, W. Stockbridge, MA 01266]. The average daily aa (average antipodal index) values as well as the daily K values from Fredericksburg (Virginia) were obtained for the experimental periods.

In Exp. 1, a total of 24 male Wistar rats, approximately 130 days of age, served as subjects, while in Exp. 2 the rats ($n=24$) were 110 days of age at the beginning of the experiment. The behavioral procedures in both experiments were designed to be as similar as possible. All rats were housed in individual plastic cages containing $\frac{1}{4}$ -in. corncob bedding. The cages were placed within one of two aluminum frames (112 cm by 125 cm by 20 cm) that supported four rows of cages (4 cages per row). One frame was wrapped along the major perimeter by 72 turns of 30 (AWG) wire (about 304 m); the other frame was not. All rats received water and Purina rat chow through the food hopper *ad libitum*. Rats were housed always less than one-half the distance to the ceiling (and overhead fluorescent lights) to minimize contributions from the ballasts (transformers); the 60-Hz field strengths were less than 0.1 mG (limits of meter).

One week before the magnetic field treatment, synchronization of external food delivery began by placing two chunks of food into the floor of the cage (Persinger, 1995a). The proportion of rats that exhibited "spontaneous" overt seizures, as defined by rearing and forepaw clonus, during the subsequent 15 min. were observed for both conditions; the observations were completed between 1845 and 1900 hr.

The treatment in Exp. 1 consisted of delivering a 7-Hz square wave (from a Model EU 81A Heath-Schlumberger function generator) for 15 min., once per day between 1730 and 1745 hr. for 14 consecutive days; the proportions of rats displaying seizures per observation period were recorded. The experimental field strengths within a given cage did not differ by more than 10 to 20 nT (limits of meter) but ranged between 100 nT and 700 nT (close to the wires) between cages within the exposure area. Maximum experimental field intensities within the control rack, placed approximately 1 m away, ranged between 10 nT and 50 nT.

The treatment in Exp. 2 involved the use of a custom-constructed simulator (built by the second author) of geomagnetic storm amplitudes and frequencies (4-channel attenuator sequencer, see Fig. 1). The ripple frequency driven by the function generator was a 7-Hz square wave, while the amplitude varied by four increasing steps (about 75 nT each) and then four (similar magnitude) decreasing steps that required a single 1-hr. period between 1700 and 1800 hr. The maximum variation of intensities between the cages ranged between 150 and 300 nT but within any given cage were only between 10 and 15 nT. The control rack received no experimental stimulation and was more than 1 m away. We did not detect any extraneous experimental fields (<0.1 mG).

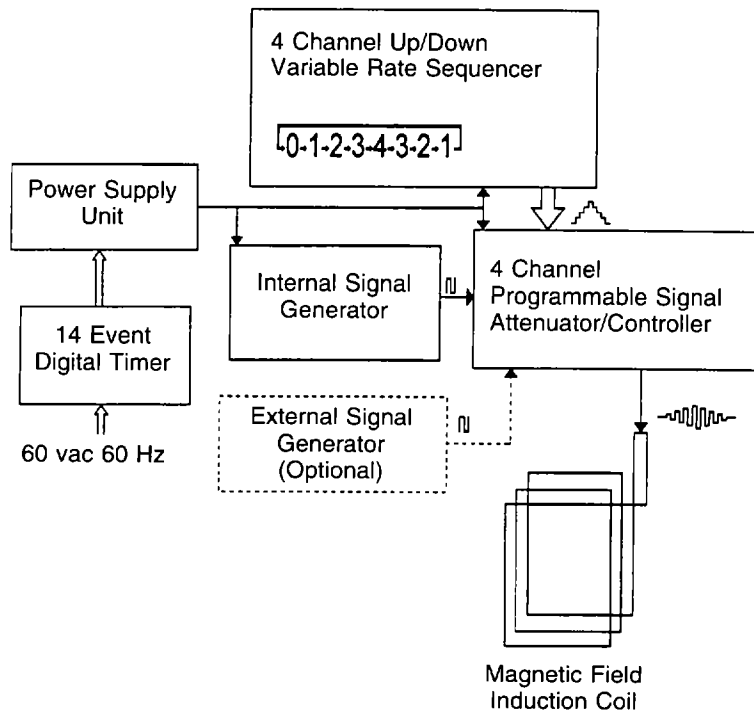


Fig. 1. Functional block diagram for the simulator of geomagnetic storms

In Exp. 3, four chronically epileptic rats were housed singly in 32-cm by 36-cm by 45-cm plastic cages. Two of these cages were each surrounded by Helmholtz coils (30 ohms each) connected in parallel to a custom-built (by S. A. Koren) output for the function generator. The output was con-

trolled by a Commodore 64 computer that activated the field (if the coil was connected to the function generator) between midnight and 0800 hr. During each successive hour the field strength was increased by four successive, equal steps (42 sec./step), asymptoted at maximum for 84 sec., and then decreased by three successive, equal steps (30 sec.) within a single 5-min. period only once per hour. This timeframe was selected because 89% of sudden storm commencements exhibit rise times between 2 min. and 6 min. (Mayaud, 1975); the average amplitude of the rise time is about 10 to 15 nT.

The time-varying component, "ripple frequency," from the function generator that was superimposed upon the intensity changes was a 7-Hz square wave. The maximum intensity of the field within the center of each coil during the peak plateau was about 60 nT to 70 nanoT while the value during the trough (no field) period was below 10 nanoT (or 0.1 milligauss). The maximum field strengths within the two "lower-intensity" cages (30 cm below the bottom of the other cages) were between 20 and 30 nT. Verification of the presence and strength of the field was made randomly at midnight three different times during the experiment.

Food was delivered to the individual cages, daily, during the 1-hr. period that preceded the onset of the night cycle (local time 1930 hr.) for 49 successive days; the occurrence was recorded of an episode (never more than one) of overt forepaw clonus and rearing during the 15-min. period after the food delivery. One week after the initiation of the daily presentation of food, the experimental magnetic field was activated during the 8-hr. period only once per week because we assumed that magnetic field-induced suppression of melatonin could require several days to recover.

RESULTS

Multiple regression analyses in Exp. 1 for the group that received the magnetic field indicated a significant decrease ($F_{1,19}=4.46$, $p<.05$; multiple $R=0.47$) in the proportion of spontaneous seizures within the 15 min. following the food presentation had the rats been exposed to the 7-Hz pulsed field (100 nT to 700 nT) for 15 min. during the previous hour. Means and standard deviations for the proportion (percentage) of seizures during the baseline and the field periods were 9.5, 7.5 and 3.6, 4.3, respectively. The control group, which was 1 m away from the fields, did not display a significantly changed proportion of seizures ($.10>p>.05$). Means and standard deviations for the baseline and the field period were 8.3, 8.3 and 4.8, 5.4, respectively. The mean and standard deviation for the ambient average, global geomagnetic activity (Mayaud's aa values) during this experiment were 21 and 13 nT.

These results appear to support the chaos/interference model (Schiff, *et al.*, 1994). Although there was no statistically significant change in the pro-

portion of displays of overt seizures within the control group, the trend for these rats (1 meter from the experimental coil) to exhibit a decrease in the proportion of seizure activity suggests a confounding variable. One candidate would be the extraneous fields from the experimental coil. Both the calculated field strength and the *post hoc* measurement within the reference area when the field was activated could have been as high as 50 nT. This value is within the range of geomagnetic activity.

Multiple regression analyses for proportions of seizures per day for the experimental and reference groups as a function of the eight successive 3-hr. K values of geomagnetic activity showed a statistically significant equation ($F_{3,17}=7.27, p<.01$) for the experimental group only. The order of entry into the equation and the partial (standardized) regression coefficients for the variables were for field presentation (-0.79), geomagnetic activity (0.64) between 2100 and 2400 hr. Universal Time (or during the period of food presentation, locally), and the geomagnetic activity between 0300 and 0600 hr. Universal Time (2300 to 0100 local time). The multiple R value was 0.75 . The facilitation of seizures during enhanced geomagnetic activity occurring during the same period as the food presentation has been reported previously (Persinger, 1995a).

The results of Exp. 2 showed no significant change either over time for the reference group or as a function of the field presentations for the experimental group. The means and standard deviations for the proportion (percentage) of the experimental group from the population that displayed overt seizures were 1.7 and 4.0 for the baseline period and 4.0 and 5.0 for the treatment period. For the reference group these values were 8.3 , 7.5 and 6.0 , 7.3 , respectively. The mean for the daily average geomagnetic activity during this period was 26 nT ($SD=17$ nT). Ambient geomagnetic activity entered the multiple regression equations for the reference group only. There was a positive correlation ($r=.61, p<.01$) between the proportion of seizures within this group and enhanced geomagnetic activity during the previous night between 0300 and 0600 hr. Universal Time. Although the chronically epileptic rats had been randomly assigned to the experimental and control groups, the explanation for the relative paucity of seizures during the baseline for the former (experimental) group is unclear.

For Exp. 3 nonparametric analyses indicated a statistically significant ($\rho=.42, p<.01$) increase in the numbers of seizures for the four rats in response to food had they been exposed to the shifting-intensity magnetic fields for eight hours during the previous night. Multiple regression analysis ($R_{2,36}=0.60, p<.001$), which employed the total numbers of rats that displayed overt forelimb clonus during the 15 min. following the food presentation and (1) absence or presence of the experimental magnetic field and (2) the aa values for the day of and each of the three days before the observation,

indicated that the numbers of seizures increased only if the experimental field had been presented during the previous night and there had been minimal geomagnetic activity three days earlier. None of the eight 3-hr. increments from the K values entered the equation. The mean for the global, average daily geomagnetic activity during the experiment was 30 nT ($SD=21$ nT).

DISCUSSION

Because the ripple frequency was held constant and was 7 Hz for all three experiments, our results focus upon the duration and intensity of the applied magnetic fields. One of the major contradictions of this area of inquiry has been the antithetical effects of (experimental) laboratory magnetic fields and (natural) geomagnetic activity upon seizure-related phenomena (Bureau & Persinger, 1995). Whereas experimental fields almost always inhibit seizure phenomena, increased geomagnetic activity enhances these phenomena.

The results of this study, although clearly neither conclusive nor elegant from the context of the symmetry of experimental design, suggest that the temporal structure of the amplitude modulations of the ripple frequencies may be an essential component of the synthetic reproduction of the effects of geomagnetic activity upon seizure-related phenomena. Constant (peak-to-peak) intensity, time-varying magnetic fields or incremental shifting fields presented just before contexts, e.g., synchronized presentation of food, that facilitate the display of overt limbic seizures may either depress or have no effect upon their overt manifestations.

However, experimental magnetic fields whose parameters most closely simulated geomagnetic activity (Exp. 3) also produced behavioral changes that were most vectorially similar to those reported from correlational studies. They primarily show a positive association between the incidence of epileptic phenomena and the intensity of geomagnetic activity (Persinger, 1995b). Presentation of these fields during the late scotophase increased the display of overt limbic seizures during the following day. The possible sensitivity of the chronic (limbic) epileptic brain to the subtle information contained within frequency modulations within weak but global geomagnetic variations must still be investigated.

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